MAJOR ARTICLE

False-Positive Gonorrhea Test Results with a Nucleic Acid Amplification Test: The Impact of Low Prevalence on Positive Predictive Value

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(See the editorial commentary by Klausner on pages 820–1)

Five false-positive gonorrhea test results from a private laboratory using a nucleic acid amplification test led to an investigation by the Hawaii State Department of Health. No unexplained increase or variation in the laboratory's positive gonorrhea test results was detected. The proportion of positive gonorrhea test results among tests performed in the population was 1.06%. The calculated positive predictive value (PPV) of the test in this setting was 60%. Documentation of sexual histories was lacking for all cases. It is imperative to obtain a sexual history for both assessing sexually transmitted disease (STD) risk and interpreting STD test results. The possibility that positive test results may be false should be considered when patients have unanticipated positive test results. Clinicians who perform STD screening tests should know the approximate prevalence of STDs in the population being screened and have a conceptual understanding of PPV and the impact of low prevalence on screening tests with imperfect specificity.

The introduction of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis* screening dramatically improved the identification of asymptomatic chlamydial infections. *C. trachomatis* isolation is technically complex, labor intensive, costly, and has relatively low sensitivity. Therefore, NAATs offer substantial benefits. In contrast, because culture for *Neisseria gonorrhoeae* has demonstrated high sensitivity and is relatively inexpensive, the use of NAATs for gonorrhea screening offers smaller benefits [1], and, in exchange for a small improvement in sensitivity, there is a decrease in specificity. Although the NAATs have very high specificity (>99%), they are imperfect. This slight compromise in specificity may become important if NAATs are used to screen individuals from a population in which there is a low prevalence of gonorrhea. In such a scenario, the positive predictive value (PPV) of the test (the proportion of positive test results that are truly positive) may be negatively impacted, leading to an unacceptably high percentage of false-positive results [2, 3].

Dual, single-swab NAATs for detection of *C. trachomatis* and *N. gonorrhoeae* have simplified testing and allowed for noninvasive urine-based screening. This is especially advantageous both because sexually transmitted disease (STD) screening services can be expanded to settings where traditional genital examinations are not performed (e.g., schools) and because at-risk populations who normally would not submit to a traditional STD examination can be screened.

However, there is also a downside to single-swab NAATs that has inadvertently been introduced: because both *C. trachomatis* and *N. gonorrhoeae* are tested si-

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multaneously by NAATs, fewer clinicians may be using culture to identify *N. gonorrhoeae* infection. Antibiotic-resistant *N. gonorrhoeae* is an emerging global public health concern [4, 5], and with diminishing numbers of *N. gonorrhoeae* isolates available for antibiotic susceptibility testing, the ability to monitor trends in antimicrobial resistance could be compromised. In addition, because the prevalence of *N. gonorrhoeae* infection is substantially lower than the prevalence of *C. trachomatis* infection, especially in most community-based settings [6], the potential for false-positive *N. gonorrhoeae* test results is elevated, because physicians who intend to screen women for *C. trachomatis* infection are also screening for *N. gonorrhoeae* infection. This report describes 5 false-positive gonorrhea test results related to the use of a NAAT that had been cleared by the US Food and Drug Administration (FDA).

CASE DEFINITION

A false-positive case was defined as a patient who had an initial positive *N. gonorrhoeae* NAAT result whose result was negative on retesting using a different *N. gonorrhoeae* NAAT and culture, who did not receive appropriate antimicrobial therapy (or for whom there was not adequate time between receipt of appropriate antimicrobial therapy and retesting to account for a negative test result), and who had a low prior probability of infection with *N. gonorrhoeae,* as determined on the basis of clinical presentation, findings of a physical examination, Gram stain findings, and sexual history.

CASE REPORTS

During the period of October 2002 through May 2003, five women (age, 19–44 years) in long-term monogamous relationships presented to the Hawaii State Department of Health (HDOH) STD clinic with unanticipated positive results of *N. gonorrhoeae* NAATs that had been performed in private sector settings. Four women had been tested by a physician, and 1 had been seen by a nurse practitioner in a physician's office. Three of the women had been screened for STDs as part of a family planning examination, and 2 of the women had been tested for STDs to evaluate symptomatic complaints that were attributed to bacterial vaginosis. Four of the 5 partners accompanied the women to the HDOH STD clinic and were also questioned and examined. None of the case subjects or partners had sexual histories or findings of a physical examination suggestive of *N. gonorrhoeae* infection, and all 9 persons (i.e., the 5 women and the 4 partners) had negative results of *N. gonorrhoeae* tests using another NAAT and culture.

Data on the cases are summarized in table 1. Each subject was asked to sign a consent form allowing the release of pertinent medical records. Relevant information from the private clinicians' medical records is included in the summary table.

INVESTIGATION

All 5 cases involved different clinicians but were linked to the same laboratory (hereafter, "laboratory A"). Laboratory A is a major private diagnostic laboratory serving a large proportion of the state's private sector health care providers. Laboratory A had been using the Cobas Amplicor CT/NG test (Roche), a PCR-based NAAT, since July 2002. Before this, they had used the LCx assay (Abbott), which had been voluntarily recalled by the manufacturer [7]. After the first discordant case was identified, the medical director of laboratory A was contacted. He reported no recent unexplained increase in the number or proportion of positive *N. gonorrhoeae* or *C. trachomatis* test results. He was unaware of any other discordant test results. He attributed the error to a possible problem with specimen labeling. An internal investigation revealed no further problems, and new protocols were developed to prevent labeling errors.

Cases 2 and 3 occurred within a 2-week period ~3 months after the first case. The temporal clustering of these cases, combined with the psychological impact on the patients and their partners, led to the initiation of the investigation. A meeting was called by the HDOH, which included HDOH personnel and the medical director of laboratory A. At this meeting, the medical director of laboratory A reported that, on a few occasions after the initial discrepant case, physician clients had questioned their patients' *N. gonorrhoeae* NAAT results. The laboratory director reported that he had contacted Roche. After the meeting, the HDOH contacted the Centers for Disease Control and Prevention (CDC) to discuss the situation and to inquire whether the CDC was aware of other false-positive results obtained by this test in other states. Two cases had been reported from Texas. The CDC notified the FDA of the discordant test results in Hawaii. The HDOH contacted Roche. Roche was aware of perceived problems with *N. gonorrhoeae* testing in Hawaii and planned to visit laboratory A but reported no knowledge of similar problems elsewhere.

Laboratory A was asked to provide the HDOH with a weekly tally of the number of *N. gonorrhoeae* and *C. trachomatis* tests performed, as well as the number of positive test results obtained, since they had started using the Cobas Amplicor CT/NG test. PPVs were calculated using standard formulas [8]. Sensitivity and specificity parameters for PPV calculations were taken from published Cobas Amplicor CT/NG test performance characteristics [9, 10] and from positivity data provided by laboratory A.

Table 1. Summary of data regarding 5 women with false-positive *Neisseria gonorrhoeae* nucleic acid amplification test (NAAT) results, October 2002–May 2003, Honolulu, Hawaii

Subject	Age, years	Reason for PC visit	PC findings and diagnosis	Treatment prescribed by PC at time of office visit	PCR-based NAAT results	Time from PC examination to HDOH clinic visit, days
1	42	Mild vaginal discharge	Presence of "clue cells," BV	Intravaginal metronidazole cream for 7 days	Positive for *N. gonorrhoeae* and *C. trachomatis*	5
2	34	Family planning	Examination findings were WNL	None	Positive for *N. gonorrhoeae*	7
3	44	"Pelvic pressure," UTI	Possible BV/UTI[f]	Intravaginal miconazole cream, oral clindamycin, oral nitrofurantoin	Initial test had "gray zone" positivity for *N. gonorrhoeae*; second test was positive for *N. gonorrhoeae*	8
4	22	Family planning	Examination findings were WNL	None	Positive for *N. gonorrhoeae*	8
5	19	Family planning	Examination findings were WNL	None	Positive for *N. gonorrhoeae*	8

NOTE. BV, bacterial vaginosis; GNED, gram-negative extracellular diplococci; GNID, gram-negative intracellular diplococci; HDOH, Hawaii State Department of Health (Honolulu); OIF, oil immersion field; PC, private clinician; PMH, past medical history; PMNs, polymorphonuclear leukocytes; STD, sexually transmitted disease; UTI, urinary tract infection; WNL, within normal limits.

[a] A prescription for cefixime (400 mg po) plus azithromycin (1 g po) was called to the pharmacy by the PC but was not accessed by the subject before her visit to the HDOH.

[b] Cervix without friability or mucopus for all women. Gram-stained endocervical swab revealed 0–4 PMNs per OIF, without GNID or GNED, for subjects 1 and 3–5; for subject 2, it yielded 10–20 PMNs per OIF, without GNID or GNED.

[c] Gram-stained urethral swab revealed 0–2 PMNs per OIF, without GNID or GNED.

[d] Transcription-mediated amplification-based NAAT was negative for *N. gonorrhoeae* and *C. trachomatis* for endocervical swab specimens obtained from all of the women and for urethral swab specimens obtained from the partners of subjects 1, 2, 4, and 5. No growth of *N. gonorrhoeae* occurred on Martin Lewis media after inoculation of endocervical and rectal swab specimens for all women and after inoculation of urethral swab specimens for the partners of subjects 1, 2, 4, and 5.

[e] Treatment was prescribed by the PC in response to positive PCR-based *N. gonorrhoeae* NAAT result.

[f] Urine culture yielded *Escherichia coli* (>10^5 organisms/mL).

RESULTS

During the period of 15 July 2002 through 15 June 2003, there were no significant detectable weekly variations in the number of *N. gonorrhoeae* or *C. trachomatis* tests performed or in the number of positive test results. The proportion of positive test results was 1.06% (177 of 16,641 tests) for *N. gonorrhoeae* and 4.13% (778 of 18,819 tests) for *C. trachomatis*.

Using published data on the Cobas Amplicor CT/NG test performance characteristics for detection of *N. gonorrhoeae* (sensitivity, 98.2%; specificity, 99.3%) [9] and *C. trachomatis* (sensitivity, 97.6%; specificity, 99.5%) [10] from samples from the endocervix of asymptomatic female subjects, the following PPVs were calculated: 60% for a positive *N. gonorrhoeae* test result, and 89.4% for a positive *C. trachomatis* test result.

Neither confirmatory testing nor retesting was performed by the private physicians, with the exception of the single "gray zone" positive result for case 3, and the medical record review revealed no documentation that sexual histories were obtained or that possible false-positive test results were discussed. The women were prescribed appropriate antimicrobial therapy and were advised to have their partners examined and treated.

DISCUSSION

A number of issues were uncovered in this investigation, including poor PPV when a test with imperfect specificity is applied to a low-prevalence population, omissions in obtaining sexual histories, and misinterpretation of screening test results. In a recent publication discussing screening tests for *C. trachomatis* and *N. gonorrhoeae* [1], the CDC recommended that (1) all positive screening tests should be considered presumptive evidence of infection; (2) an additional test should be considered after a positive screening test result if a false-positive screening test result would lead to substantial adverse medical, social, or psychological impact for a patient; and (3) consideration should be given to routine performance of an additional test after a positive screening test result if the PPV is considered low (e.g., <90%).

Medications taken by patient before HDOH clinic examination	Data from HDOH clinic visit			
	Sexual history and findings			
	Subject	Male partner	Treatment	Laboratory test results
Intravaginal metronidazole for 5 days before examination[a]	7-Year-long monogamous relationship; no PMH of STD; examination findings were WNL[b]	Asymptomatic; 7-year-long monogamous relationship; examination findings were WNL[c]	Deferred pending test results	Negative[d]
Cefixime (400 mg po) 2 h before examination[e]	6-Year-long monogamous relationship; no PMH of STD; examination findings were WNL[b]	Asymptomatic; 6-year-long monogamous relationship; examination findings were WNL[c]	Deferred for partner pending test results	Negative[d]
Oral clindamycin, nitrofurantoin for 4 days before examination, and intravaginal miconazole for 7 days before examination	10-Year-long monogamous relationship; no PMH of STD; examination findings were WNL[b]	Not seen	Ceftriaxone (125 mg im), azithromycin (1 g po)	Negative[d]
Cefixime (400 mg po) 5 h before examination[e]	4-Year-long monogamous relationship; no PMH of STD; examination findings were WNL[b]	Asymptomatic; 4-year-long monogamous relationship; examination findings were WNL[c]	Deferred for partner pending test results	Negative[d]
None	1.5-Year monogamous relationship; 1 lifetime partner; no PMH of STD; examination revealed asymptomatic candidiasis[b]	Asymptomatic; 1.5-year-long monogamous relationship; examination findings were WNL[c]	*N. gonorrhoeae* infection treatment deferred pending test results; tercon-azole intravaginal cream	Negative[d]

Although the *N. gonorrhoeae* NAATs have excellent performance characteristics, their specificity is not perfect. Of particular note, the Cobas Amplicor CT/NG test is known to cross-react with certain nongonococcal *Neisseria* species [1, 9]. However, this reportedly can be controlled using a "gray zone" retesting algorithm, which was used by laboratory A. This algorithm involves establishing a large equivocal zone and retesting specimens with equivocal results. Cross-reactive species tend to give negative results when retested [9].

When *N. gonorrhoeae* NAATs are applied to a population with a low prevalence of gonorrhea (e.g., older women or female subjects in long-term, mutually monogamous relationships), the tests' PPV could be unacceptably low. For the population served by laboratory A, the PPV for the *N. gonorrhoeae* NAAT was only 60%.

However, if the population being screened has a high prevalence of gonorrhea, the PPV of the test will be enhanced, and the NAAT may yield important population benefits that would more than outweigh the risk associated with false-positive results. In a recently published household survey of Baltimore residents, a prevalence of asymptomatic gonorrheal infection of 5.3% was detected using a urine-based NAAT [11]. The calculated PPV in this scenario approached 90%.

Figure 1 illustrates the relationships between test specificity, prevalence of infection in the population being tested, and the PPV of a test. For a test with a given specificity, the PPV will vary directly with the prevalence of infection in the population being tested: the higher the prevalence of the infection in the population, the higher the PPV; the lower the prevalence of infection in the population, the lower the PPV. In addition, a test with higher specificity will have a higher PPV than will a test with lower specificity when applied to a population with a given prevalence of infection. As the specificity of a test approaches 100%, the PPV will also be enhanced, and, by definition, a test with 100% specificity will yield no false-positive results—therefore, the PPV of the test would also be 100%.

Although the CDC has comprehensive guidelines in place for chlamydia screening, which include routine annual screening of all sexually active female persons aged ⩽25 years, as well as older women with "risk factors" (e.g., those who have a new sex partner and those with multiple sex partners) [12], screening recommendations for gonorrhea are less comprehensive. In their most recent STD treatment guidelines, the CDC only recommends screening asymptomatic women "at high risk" for gonorrhea, without explicitly defining "high risk" [12]. The need for comprehensive *N. gonorrhoeae* screening guidelines was the subject of a recent meeting of "external consultants" convened by the CDC [13]. There was consensus that the wide geographic variability in gonorrheal prevalence will "likely require different screening approaches at various prevalence levels" (p. 8). Of particular note, the consultants agreed that *N. gonorrhoeae* screening should be linked to *C. trachomatis* screening, but only when appropriate, and that "gonorrhea screening is not necessary in all populations targeted for chlamydia screening" [13, p. 8]. The US Preventive Services Task Force also recommends that "high-risk" women be screened for gonorrhea. They define "high-risk" women as commercial sex workers, persons with a history of repeated gonorrheal

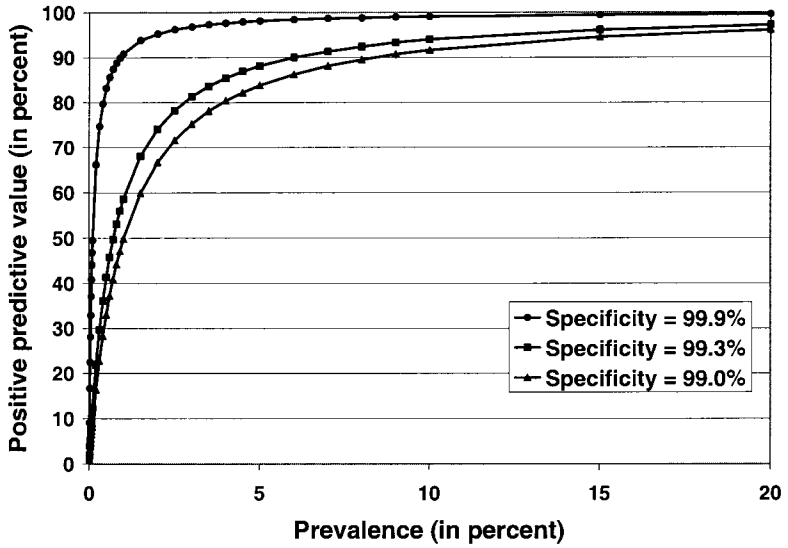

Figure 1. Positive predictive values for a test with a sensitivity of 98.2% across a range of values of specificity and prevalence of infection.

infections, and women aged <25 years who have had ⩾2 sex partners in the past year [14].

With the single-swab *C. trachomatis/N. gonorrhoeae* NAATs becoming more widely used, screening practices for *N. gonorrhoeae* are being influenced by *C. trachomatis* screening recommendations. In this case series, 2 of the 3 asymptomatic women met *C. trachomatis* screening recommendation criteria (i.e., they were sexually active and ⩽25 years of age), while 1 had no indications for STD screening (i.e., she was asymptomatic, aged >30 years, and in a 6-year-long mutually monogamous relationship).

All 5 patients in this study were in long-term, mutually monogamous relationships. In 3 cases, the tests were performed for asymptomatic women undergoing family planning examinations; in 2 cases, the tests were performed for women with symptoms of vaginal discharge who had received a diagnosis of bacterial vaginosis. Although symptomatic vaginal discharge may justify STD testing, it is imperative that physicians obtain sexual histories from the patients they test; otherwise, interpretation of the test results may be compromised.

The psychosocial effect of a false-positive STD test result should not be underestimated [3]. All 5 patients, as well as the partners we examined, were emotionally impacted. Patient 3 reported evicting her partner from their shared residence. Patient 1 terminated a several-year-long association with her health care provider after learning that her test results were in error.

A recently published survey of US physicians documented deficiencies in STD screening practices and recommended that health departments collaborate with private physicians to improve the quality of STD-related patient care [15]. Enhancing the prevalence of STD screening by physicians is important, but selective screening must also be incorporated. In addition, physicians must apply clinical judgment in the interpretation of positive screening test results.

Physician deficiencies in obtaining sexual histories have been well recognized [16, 17]. Explanations include embarrassment [18], anxiety [19], lack of time [20], and perceptions of being ill prepared [21]. Ongoing efforts have been made to address these issues so that obtaining a sexual history becomes a routine part of a patient-physician encounter [19, 21, 22].

To address these issues, an educational approach was undertaken. Laboratory A agreed to append a notification with all positive *N. gonorrhoeae* NAAT results mentioning the possibility of false-positive test results, along with instructions to access a more comprehensive advisory (including laboratory-specific positivity data for *N. gonorrhoeae* and *C. trachomatis* and PPV calculations) posted on their Web site. On the basis of the HDOH's experience, the state's other major private sector diagnostic laboratory ("laboratory B") agreed to take a proactive approach and append a notification to their positive *N. gonorrhoeae* NAAT results, with similar instructions to access a more comprehensive advisory on their Web site. In addition, the HDOH published a physician advisory reminding physicians of the importance of obtaining sexual histories, explaining the concept of PPV and the impact of low prevalence, and delineating the appropriate application and interpretation of STD screening tests. This was mailed to all licensed physicians in the state and was also posted on the HDOH Web site [23].

False-positive test results should be considered when patients have unanticipated positive results of screening tests for *N. gonorrhoeae* or *C. trachomatis,* especially in cases in which the sexual history data or clinical findings do not support the laboratory results. Obtaining a sexual history is imperative both

for assessing STD risk and for interpreting screening test results. Physicians who do not obtain sexual histories are at risk both of missing patients who practice unsafe behaviors who should be screened for STDs and of misinterpreting screening test results when they are applied.

We recommend that all physicians obtain the sexual histories of their patients, be familiar with current STD screening recommendations, and selectively apply STD screening tests. In situations in which positive test results are not supported by sexual history data or clinical findings, we recommend retesting the patient using a different NAAT or culture (to confirm a gonococcal infection). A decision to delay treatment pending retest confirmation should be made in consultation with the patient. The CDC currently advises that patients "be counseled regarding prompt treatment after a positive screening test because an additional test might be falsely negative" [1, p. 14] and notes that, "because treatments for *C. trachomatis* and *N. gonorrhoeae* are safe and relatively inexpensive, the person might wish to receive and complete treatment while additional testing is being done, or even if the additional test is negative" [1, p. 16].

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